Exploration of Durotaxis through Agent-Based Modeling

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April 24, 2015

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Acknowledgements

I would like to acknowledge Dr. Keith Gooch, James Reinhardt and Alex Clark for their significant contributions to my project. Additionally, I would like to thank the Ohio State University College of Engineering for funding my project.

Background

Cell migration and remodeling of the ECM are involved in many biological processes, including development[1], wound healing[2], fibrotic pathologies[3] and of particular interest, tissue engineering[4,5]. Although the importance of these processes has been recognized, a complete understanding of the mechanisms governing cell migration and remodeling of the ECM remain unclear.

Duortaxis, the migration of cells in the direction of increasing ECM stiffness, is one specific phenomenon that has been observed experimentally, but remains poorly understood[6]. Gooch et al. replicated durotaxis in an agent-based model (ABM), which simulated cell and ECM interactions based on a complex set of rules[7]. Although the complexity of the model allowed for many insights into cell migration and ECM remodeling, it also made it difficult to gain an intuitive understanding of the specific phenomenon of duortaxis. In order to gain this intuitive understanding of durotaxis, creating a simplistic ABM to explore durotaxis was proposed.

Objectives

The objectives of this project were to:

1.) Develop an ABM using *Netlogo* that can capture durotaxis in its most fundamental form; and

2.) Determine what rules are essential or non-essential in capturing durotaxis

Methodology

Netlogo, an ABM environment, was used to develop a model for exploring durotaxis. An ABM consists of autonomous agents that interact based on a set of rules. These interactions between agents give rise to emergent behavior, defined as the collective behavior of the system resulting from lower level interactions between agents[8]. ABM provides the advantage of being able to model the dynamics of cell migration whereas other modeling techniques, such as finite element analysis, may only be able to model one specific interaction at one point in time.

The model was created using a 'construction' approach. That is, the simplest model possible that captures processes essential for durotaxis was constructed in order to determine the ability of the simulated cell to exhibit durotaxis. Once durotaxis was observed in its most fundamental form, features of the model were sequentially varied in order to determine specific rules that were essential or non-essential to durotaxis.

Model Description

The model contained three agents:

1.) Cell

2.) Left pseudopod

3.) Right pseudopod

Figure 1: Model agents

The model contained four sets of rules:

1.) Pseudopod extension

a. Pseudopod extension describes the process in which a cell extends a pseudopod and grabs the ECM. The three variations for this rule can be seen in Table 1. Instant extension was determined to be the control. Other variations were created in order to determine how essential or non-essential the specifics of pseudopod extension were to durotaxis.

Table 1: Pseudopod extension rules

* Indicates control

Extension Type	After Retraction?	Extend Length
Instant *	Instantly begin extension	5
Random Delay	Each iteration, pseudopod has 20% of beginning extension, otherwise wait	5
Random Length	Instantly begin extension	2 - 5

2.) Pseudopod detachment

a. Pseudopod detachment describes the process in which a cell releases its hold on the ECM. The two variations for this rule can be seen in Table 2. Length detachment was determined to be the control. Other variations were created in order to determine how essential or non-essential the specifics of pseudopod detachment were to durotaxis.

Table 2: Pseudopod detachment rules

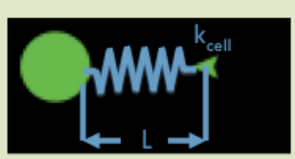
* Indicates control

Detachment Type	After Retraction?
Length *	Detach once pseudopod center reaches edge of cell diameter
Random Length	Detach at random distance of 0-2 from edge of cell diameter

3.) Cell-pseudopod interaction

a. The force required to extend a pseudopod was considered negligible and assumed to be zero. On the other hand, the force required to retract a pseudopod had two modeling variations as seen in Table 3.

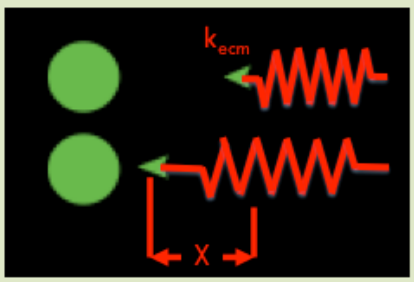
Table 3: Cell-pseudopod interaction rules

Interaction Type	Physical Interpretation	Mathematical Interpretation
Constant		$F_{retract} = constant$
Spring		$F_{retract} = k_{cell} * L$

4.) Pseudopod-ECM interaction

a. The ECM resisted the pull of the cell on its pseudopod as shown in Table 4. In order to create a stiffness gradient, k_{ecm} increased from left to right. Therefore, the right pseudopod always experienced a greater resistance from the ECM than the left pseudopod.

Table 4: Pseudopod-ECM interaction rules

Interaction Type	Physical Interpretation	Mathematical Interpretation
Spring		$F_{ecm} = k_{ecm} * X$

A durotactic index (DI) was defined in order to quantify durotaxis[9]. The DI was defined as follows:

$$Durotactic\ Index = \frac{d_{right} - d_{left}}{d_{total}} \quad \text{(Equation 1)}$$

$$d = displacement$$

The DI could range between -1 and 1. DI = 1 indicates perfect durotaxis, DI = 0 indicates no net displacement and DI = -1 indicates perfect reverse durotaxis.

Results

The constant force control experiment results can be seen in Figure 2. For this experiment, durotactic index increased as gradient slope increased. Additionally, at minimal stiffness gradients, the cell had no preference for one direction. These results achieved the first objective to develop a model that captures durotaxis in its most fundamental form.

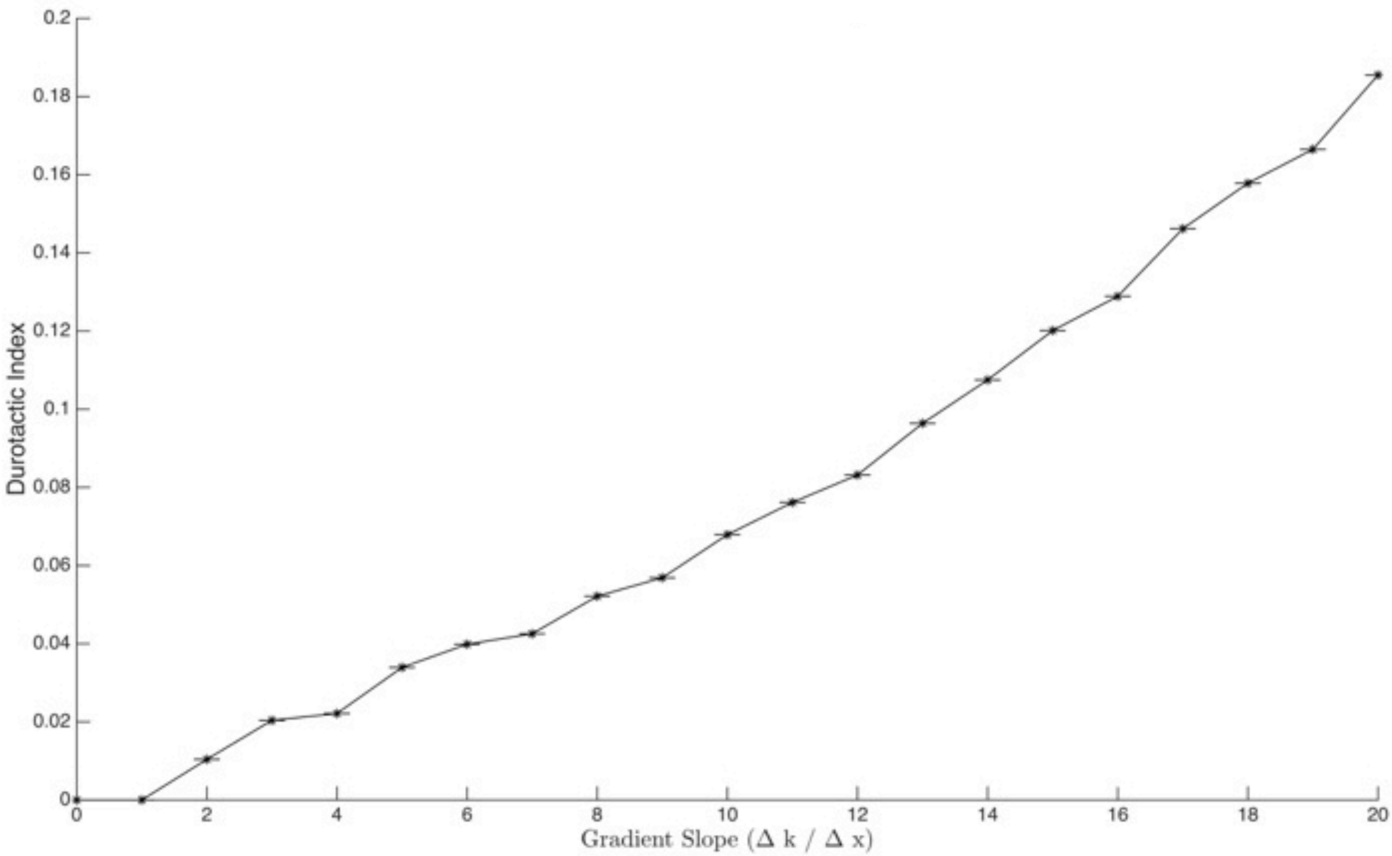


Figure 2: Constant force control experiment

The constant force control experiment plus variations can be seen in Figure 3. Both the control experiment and random delay extension variation showed similar results. Additionally, the random length extension and random length detachment seemed to exhibit durotaxis to a slightly lesser degree.

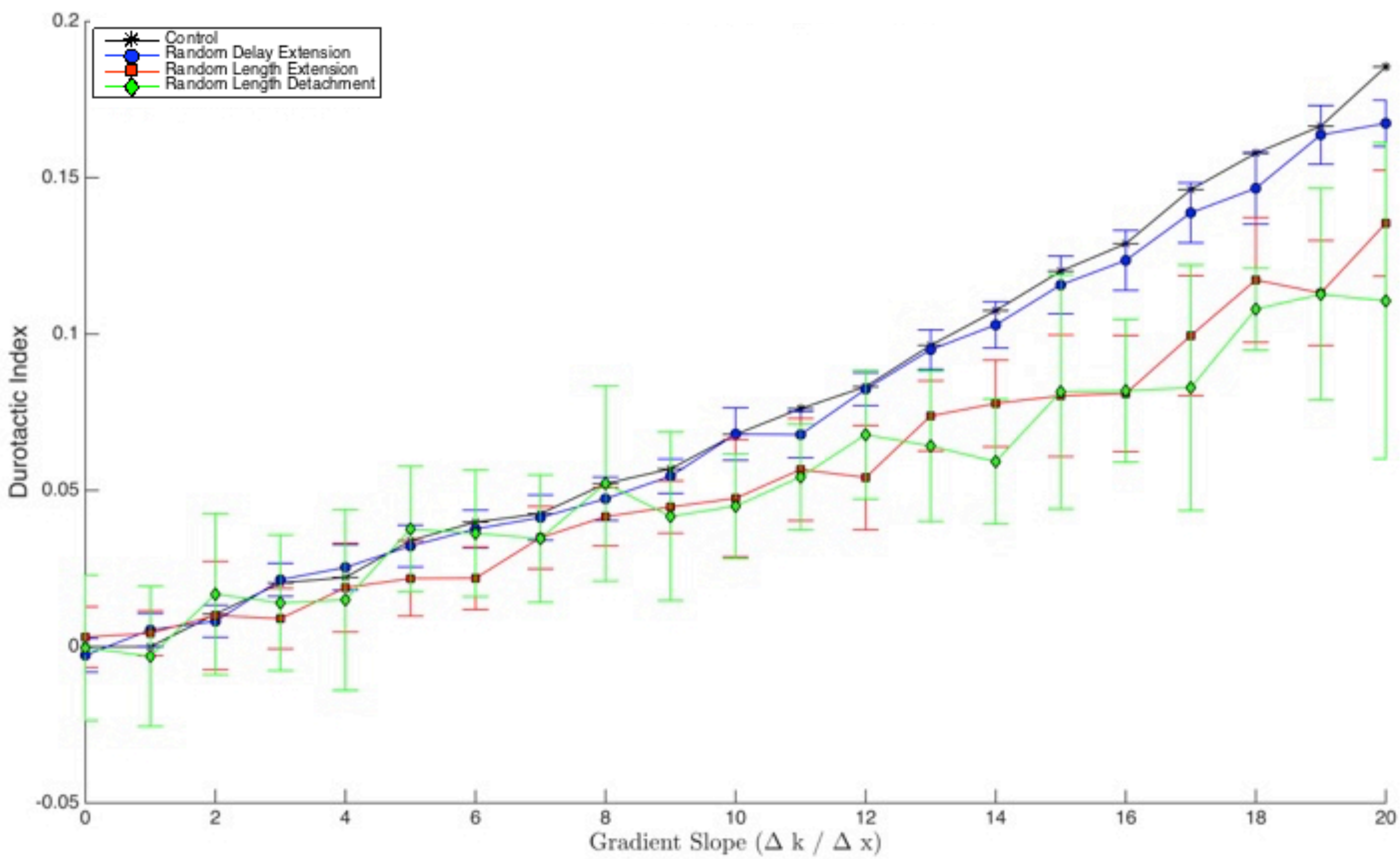


Figure 3: Constant force experiments

Each control experiment was run once because it was a deterministic simulation. Each variation experiment was run 10 times and the mean +/- the standard deviation was plotted.

The spring force control experiment results can be seen in Figure 4. Similar to the constant force control experiment, durotactic index increased as gradient slope increased. Additionally, at minimal stiffness gradients, the cell had no preference for one direction. Once again, these results achieved the first objective to develop a model that captures durotaxis in its most fundamental form.

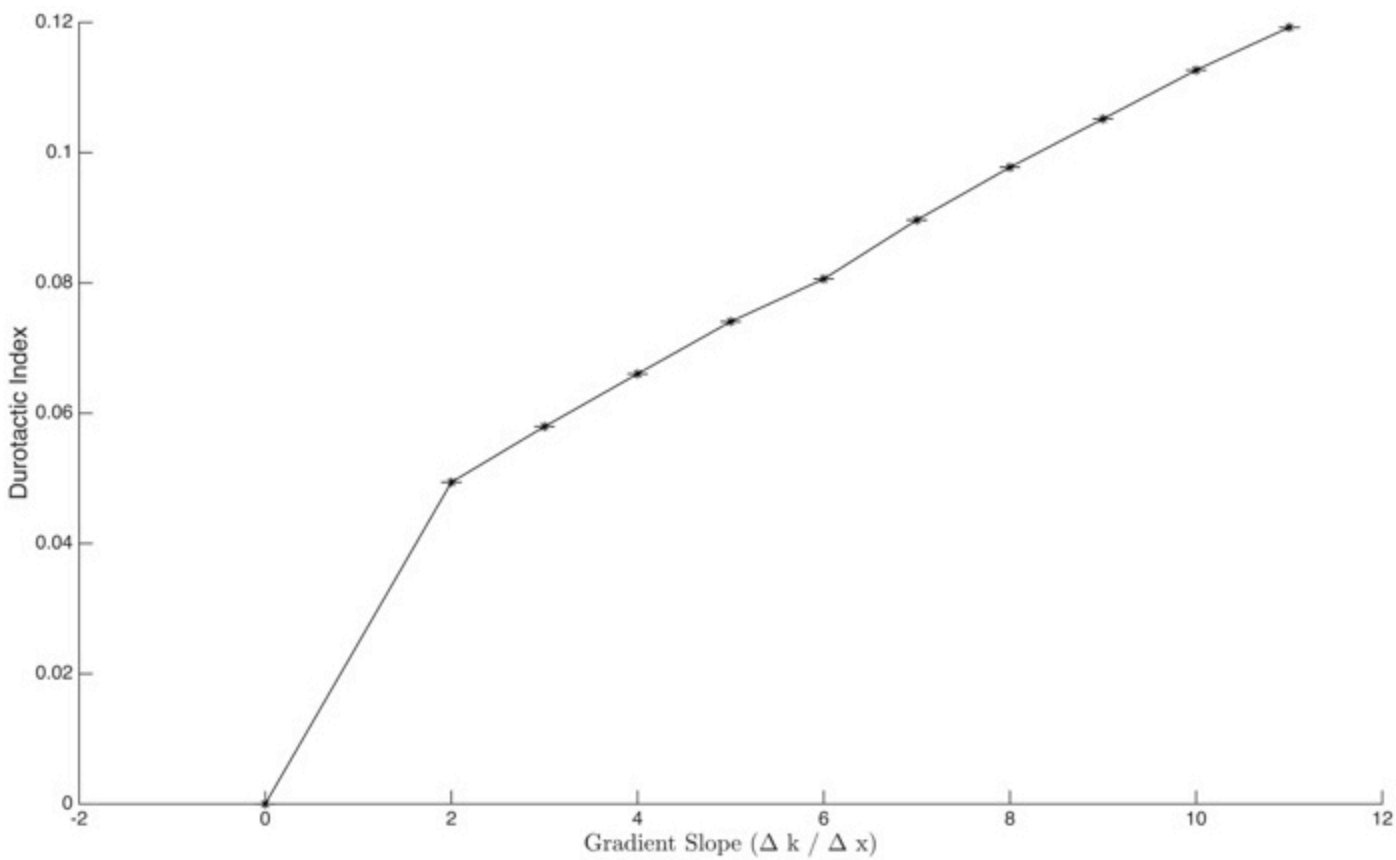


Figure 4: Spring force control experiment

An outlier was removed from this plot and will be mentioned in the limitations. Additionally, the plot was truncated at a gradient slope equal to 11 for reasons that will also be mentioned in the discussion.

The spring force control experiment plus variations can be seen in Figure 5. The control experiment showed increasing durotactic index with increasing gradient slope up until a gradient slope equal to 11. As the gradient slope increased further, the durotactic index dropped off. The random delay extension variation showed results consistent with durotaxis. Both the random length extension and random length detachment seemed to exhibit negligible durotaxis.

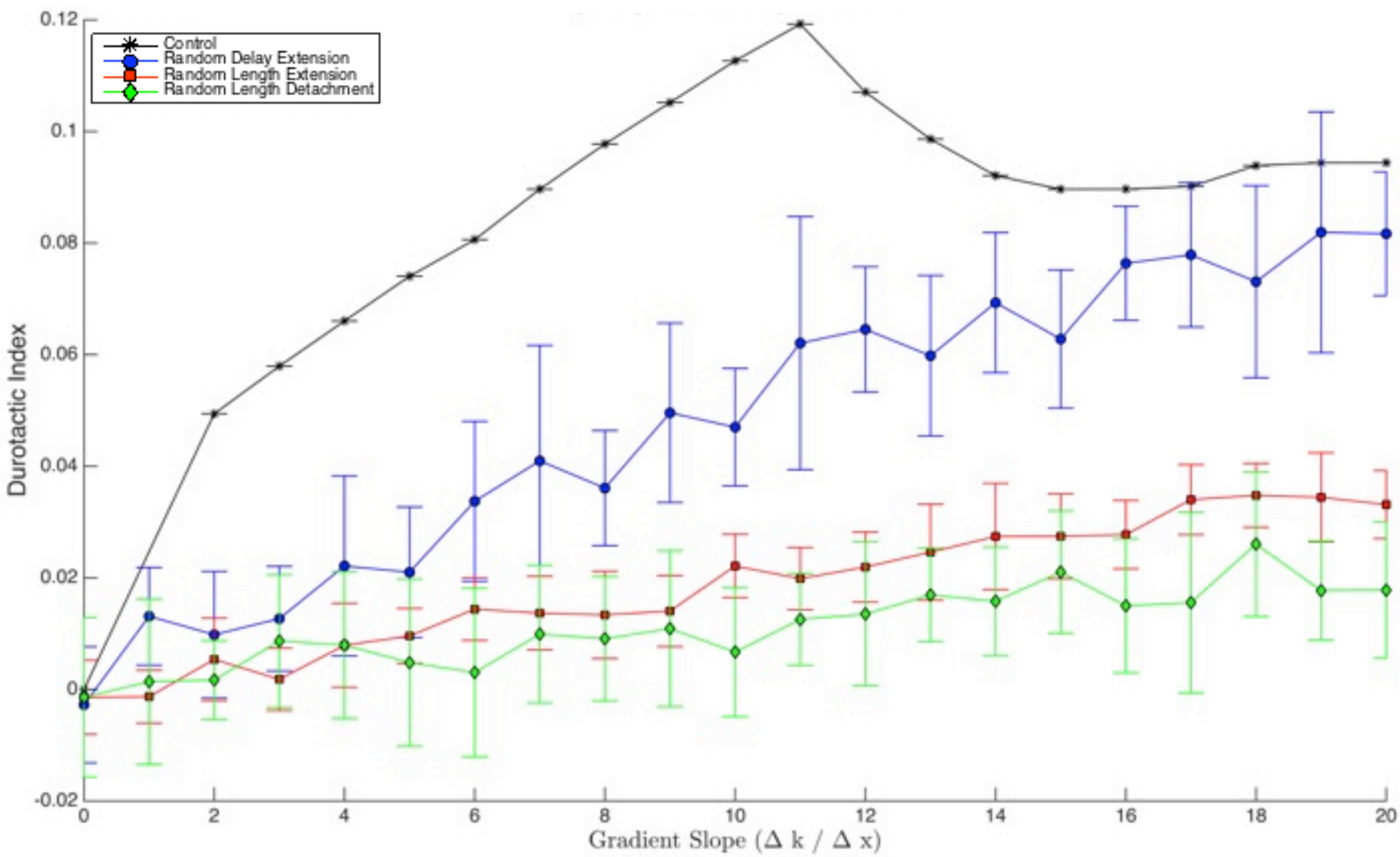


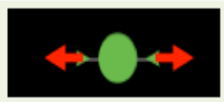
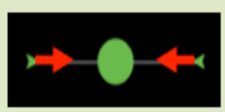
Figure 5: Spring force experiments

Each control experiment was run once because it was a deterministic simulation. Each variation experiment was run 10 times and the mean +/- the standard deviation was plotted.

Discussion

For constant force experiments, a net force on the cell was only present while pseudopods were in opposite states. The magnitude of the net force was always equal. Table 5 shows the direction of net force on the cell for all four possible states of the system. It can thus be seen that in order for the cell to exhibit durotaxis, the right pseudopod must spend a greater time in retraction than the left pseudopod. This was a direct result of the right pseudopod experiencing a greater resistance from the ECM.

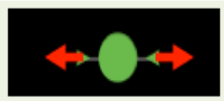
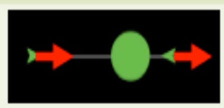
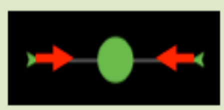
Table 5: Constant force cell states

Psuedopod L / Pseudopod R	Extend / Extend		0
Psuedopod L / Pseudopod R	Extend / Retract		+
Psuedopod L / Pseudopod R	Retract / Extend		-
Psuedopod L / Pseudopod R	Retract / Retract		0

The constant force model was robust and did not require one specific rule for pseudopod extension or detachment in order to observe durotaxis. Therefore, durotaxis can be promoted by varying cellular or ECM parameters that can cause the cell to pull on its pseudopods with a constant force.

For spring force experiments, a net force on the cell was present while pseudopods were in opposite states and while both pseudopods were retracting. Table 6 shows the direction of net force on the cell for all four possible states of the system. It can thus be seen that both the right pseudopod spending a greater time in retraction than the left pseudopod and the net force on the cell to the right during simultaneous retraction allowed durotaxis to occur. Once again, this was a direct result of the right pseudopod experiencing a greater resistance from the ECM.

Table 6: Spring force cell states

Psuedopod L / Pseudopod R	Extend / Extend		0
Psuedopod L / Pseudopod R	Extend / Retract		+
Psuedopod L / Pseudopod R	Retract / Extend		-
Psuedopod L / Pseudopod R	Retract / Retract		+

The spring force model was sensitive to variations in the rules for pseudopod extension and detachment. Consequently, a cell retracting its pseudopods with a spring force mechanism may have difficulty moving up a stiffness gradient.

The cell also encountered a maximum stiffness during spring force experiments. The spring force control experiment reached its maximum stiffness during simulations with a gradient slope of 11 or greater. The maximum stiffness was the point at which the cell could no longer fully retract its pseudopod. The right pseudopod reached the maximum stiffness before the left pseudopod, explaining why the durotactic index fell off. This observed maximum stiffness predicts that cells placed on an extremely stiff substrate may not be capable of migrating. Therefore, increasing ECM stiffness can be used as a mechanism to slow or halt cell migration.

Limitations

The outlying point from the spring force control experiment can be seen in Figure 6. This outlier was the result of a limitation in the model. Since a discrete time step was used to calculate cell and pseudopod position, a small stiffness gradient resulted in both pseudopods retracting to the cell edge during the same time step. This meant that both pseudopods extended and retracted in complete synchrony, leading to perfect duortaxis because a net force moving the cell to the right was the only force that could arise.

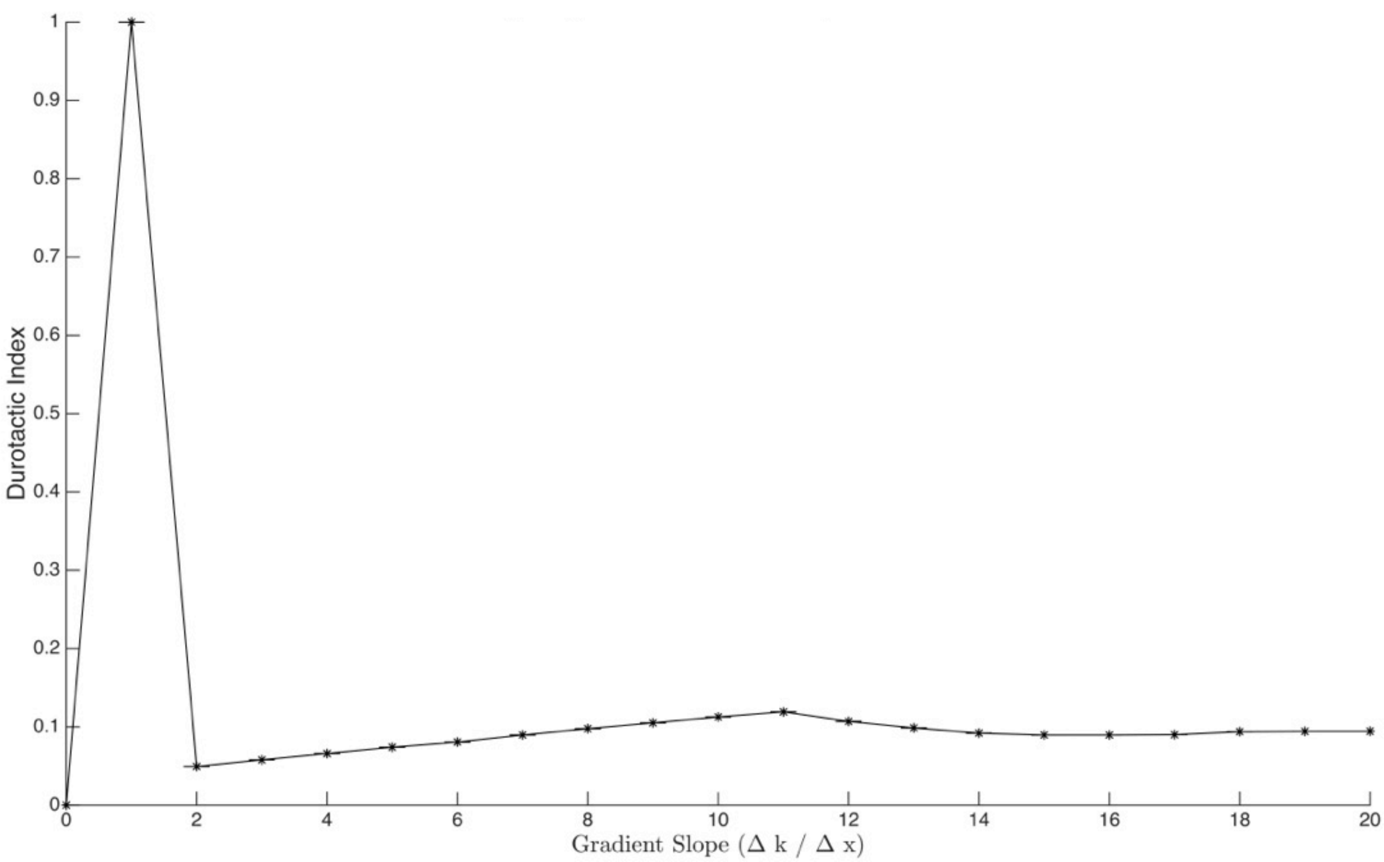


Figure 6: Spring force control experiment with outlier

Future Directions

Although an intuitive understanding of durotaxis was developed through this project, implementing a more realistic mechanism for pseudopod extension and retraction may allow for more applicable insights. Odde et al. proposed a stochastic motor-clutch model for these processes that has been experimentally validated[10]. In the model, myosin motors retract the F-actin bundles retrogradely. The molecular clutches and compliant substrate resist the pull from the myosin motors. Exploring the interactions between different aspects of the motor-clutch model could allow for a more accurate understanding of durotaxis.

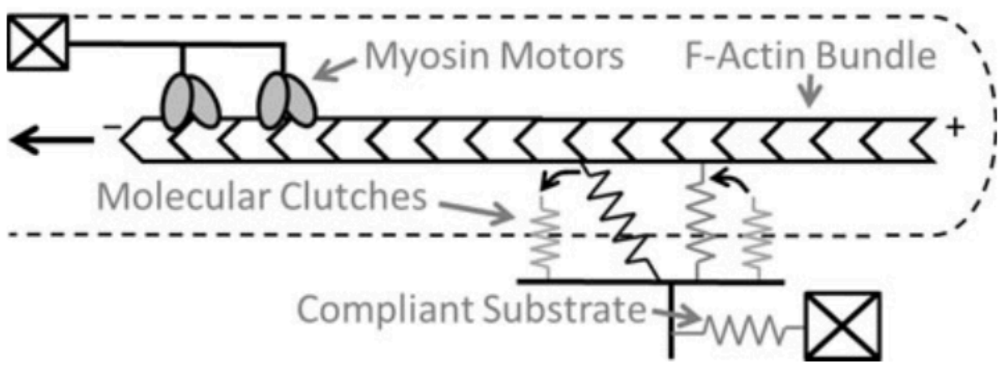


Figure 7: Motor-clutch model

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